08-01-00

BOX A

Practitioner's Docket No. U 012799-1 *PATENT*

Preliminary Classification:
Proposed Class:
Subclass:

NOTE: "All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.'" M.P.E.P. Section 601, 7th ed.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of
Inventor(s):

1. ANAND C. BURMAN
2. SUDHANAND PRASAD
3. RAMA MUKHERJEE
4. MANU JAGGI
5. ANU T. SINGH
6. ARCHNA MATHUR

WARNING: *37 C.F.R. Section 1.41(a)(1) points out:*

"(a) A patent is applied for in the name or names of the actual inventor or inventors.

(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by Section 1.63, except as provided for in Section 1.53(d)(4) and Section 1.63(d). If an oath or declaration as prescribed by Section 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to Section 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in Section 1.17(I) is filed supplying or changing the name or names of the inventor or inventors."

For (title): BOMBSIN ANALOGS FOR TREATMENT OF CANCER

CERTIFICATION UNDER 37 C.F.R. 1.10*
*(Express Mail label number is **mandatory**.)*
(Express Mail certification is optional.)

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on this date July 31, 2000, in an envelope as "Express Mail Post Office to Addressee", m ailing Label Number EL386270297US+, addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

GERALDINE MARTI
(type or print name of person mailing paper)

Geraldine Marti

Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

***WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).*

*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

1. Type of Application

This new application is for a(n)

(check one applicable item below)

[ ] Original (nonprovisional)
[ ] Design
[ ] Plant

WARNING: ***Do not*** *use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application.*

WARNING: ***Do not*** *use this transmittal for the filing of a provisional application.*

NOTE: If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

[ ] Divisional.
[ ] Continuation.
[X] Continuation-in-part (C-I-P).

2. Benefit of Prior U.S. Application(s) (35 U.S.C. Sections 119(e), 120, or 121)

NOTE: A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. Section 112. Each prior application must also be:

(I) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America; or

(ii) Complete as set forth in Section 1.51(b); or

(iii) Entitled to a filing date as set forth in Section 1.53(b) or Section 1.53(d) and include the basic filing fee set forth in Section 1.16; or

(iv) Entitled to a filing date as set forth in Section 1.53(b) and have paid therein the processing and retention fee set forth in Section 1.21(l) within the time period set forth in Section 1.53(f).

37 C.F.R. Section 1.78(a)(1).

NOTE If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-I-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

WARNING: *When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application* ***must*** *be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. Section 1.78(a)(3).*

[X] The new application being transmitted claims the benefit of prior U.S. application(s). Enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed

A. Required for Filing Date under 37 C.F.R. Section 1.53(b) (Regular) or 37 C.F.R. Section 1.153 (Design) Application

__21__ Pages of Specification
__3__ Pages of Claims
____ Sheets of Drawing

WARNING: ***DO NOT*** *submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to Section 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. For comments on proposed then-new 37 C.F.R. 1.84, see Notice of March 9, 1988 . (1990 O.G. 57-62).*

NOTE: *"Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8 inch) down from the top of the page. . ." 37 C.F.R. Section 1.84(c)).*

(complete the following, if applicable)

[ ] The enclosed drawing(s) are in color, and there is also attached a "PETITION TO ACCEPT COLOR DRAWING(S)." 37 C.F.R. Section 1.84(b).

[ ] Formal
[ ] Informal

B. Other Papers Enclosed

____ Pages of declaration and power of attorney
__1__ Pages of Abstract
____ Other

4. Additional Papers Enclosed

[ ] Amendment to claims

[ ] Cancel in this applications claims _____ before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

[ ] Add the claims shown on the attached amendment. (Claims added have been numbered consecutively following the highest numbered original claims.)

[X] Preliminary Amendment
[X] Information Disclosure Statement (37 C.F.R. Section 1.98)
[X] Form PTO-1449 (PTO/SB/08A and 08B)
[X] Citations
[ ] Declaration of Biological Deposit
[X] Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.
[ ] Authorization of Attorney(s) to Accept and Follow Instructions from Representative
[ ] Special Comments
[ ] Other

5. Declaration or Oath (including power of attorney)

NOTE: A newly executed declaration is not required in a continuation or divisional application provided the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under Section 1.47 then a copy of that declaration must be filed accompanied by a copy of the decision granting Section 1.47 status or, if a nonsigning person under Section 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 C.F.R. Section 1.63(d)(1)-(3).

NOTE: A declaration filed to complete an application must be executed, identify the specification to which it is directed, identify each inventor by full name, including the family name, and at least one given name without abbreviation together with any other given name or initial, and the residence, post office address and country of citizenship of each inventor, and state whether the inventor is a sole or joint inventor. 37 C.F.R. Section 1.63(a)(1)-(4).

NOTE: A The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by Section 1.62, except as provided for in Section 1.53(d)(4) and Section 1.63(d). If an oath or declaration as prescribed by Section 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to Section 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in Section 1.17(I) is filed supplying or changing the name or names of the inventor or inventors. 37 C.F.R. Section 1.41(a)(1).

[ ] Enclosed

Executed by

(check **all** *applicable boxes)*

[ ] inventor(s).
[ ] legal representative of inventor(s). 37 C.F.R. Section 1.42 or 1.43.
[ ] joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.

[ ] This is the petition required by 37 C.F.R. Section 1.47 and the statement required by 37 C.F.R. Section 1.47 is also attached. *See* item 13 below for fee.

[X] Not Enclosed.

NOTE: *Where the filing is a completion in the U.S. of an International Application, or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.*

[X] Application is made by a person authorized under 37 C.F.R. 1.41 on behalf of *all* the above named inventor(s).

(The declaration or oath, along with the surcharge required by 37 C.F.R. Section 1.16(e), can be filed subsequently).

[ ] Showing that the filing is authorized.
(not required unless called into question. 37 C.F.R. Section 1.41(d))

6. Inventorship Statement

WARNING: *If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.*

The inventorship for all the claims in this application are:

[ ] The same.

or

[ ] Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,
[ ] is submitted.
[ ] will be submitted.

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of $130.00 required by 37 C.F.R. Section 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 C.F.R. Section 1.52(d).

[X] English
[ ] Non-English

[ ] The attached translation includes a statement that the translation is accurate. 37 C.F.R. Section 1.52(d).

8. Assignment

[X] An assignment of the invention to DABUR RESEARCH FOUNDATION

[ ] is attached. A separate [ ] "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or [ ] FORM PTO 1595 is also attached.
[X] will follow.
[ ] has been recorded at Reel _____, Frame _____ on _____

NOTE. "If an assignment is submitted with a new application, send two separate letters-one for the application and one for the assignment" Notice of May 4, 1990 (1114 O.G. 77-78).

WARNING: *A newly executed "STATEMENT UNDER 37 C.F.R. Section 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O.G. 62-64.*

9. Certified Copy

Certified copy(ies) of application(s)

INDIA	147/DEL/2000	24TH FEBRUARY 2000
Country	Appln. no.	Filed
Country	Appln. no.	Filed
Country	Appln. no.	Filed

from which priority is claimed

[ ] is (are) attached.
[X] will follow.
[ ] was filed in parent application _____

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 C.F.R. Section 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application, then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 C.F.R. Section 1.16)

A. [X] Regular application

CLAIMS AS FILED					
Claims	Number Filed	Basic Fee Allowance	Number Extra	Rate	Basic Fee 37 C.F.R. Section 1.16(a) $690.00
Total Claims (37 C.F.R. Section 1.16(c))	20	- 20 =	x	$ 18.00	
Independent Claims (37 C.F.R. Section 1.16(b))	2	- 3 =	x	$ 78.00	
Multiple Dependent Claim(s), if any (37 C.F.R. Section 1.16(d))			+	$260.00	

[ ] Amendment cancelling extra claims is enclosed.
[ ] Amendment deleting multiple-dependencies is enclosed.
[ ] Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 C.F.R. Section 1.16(d).

Filing Fee Calculation $ 690.00

B. [ ] Design application
($310.00--37 C.F.R. Section 1.16(f))
Filing Fee Calculation $_____

C. [ ] Plant application
($480.00--37 C.F.R. Section 1.16(g))
Filing Fee Calculation $_____

11. Small Entity Statement(s)

[ ] Statement(s) that this is a filing by a small entity under 37 C.F.R. Section 1.9 and 1.27 is (are) attached.

WARNING: *"Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under Section 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under Section 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent or includes a copy of the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this Section." 37 C.F.R. Section 1.28(a)(2).*

WARNING: *"Small entity status must not be established when the person or persons signing the . . . statement can* ***unequivocally*** *make the required self-certification." M.P.E.P. Section 509.03, 6th ed., rev. 2, July 1996 (emphasis added).*

(complete the following, if applicable)

[ ] Status as a small entity was claimed in prior application _____, filed on _____ from which benefit is being claimed for this application under:

35 U.S.C. Section
- [ ] 119(e) - provisional,
- [ ] 120 - continuation,
- [ ] 121 divisional,
- [ ] 365(c) - PCT,

and which status as a small entity is still proper and desired.

[ ] A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of **A**, **B** or **C** above) $_____

NOTE: Any excess of the full fee paid will be refunded if a small entity status is established refund request are filed within 2 months of the date of timely payment of a full fee. The two-month period is not extendable under Section 1.136. 37 C.F.R. Section 1.28(a).

12. Request for International-Type Search (37 C.F.R. Section 1.104(d))

(complete, if applicable)

[ ] Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made at This Time

[ ] Not Enclosed

[ ] No filing fee is to be paid at this time.
(This and the surcharge required by 37 C.F.R. Section 1.16(e) can be paid subsequently.)

[X] Enclosed

[X]	Filing fee	$ 690.00
[ ]	Recording assignment ($40.00; 37 C.F.R. Section 1.21(h)) (See attached "COVER SHEET FOR ASSIGNMENT ACCOMPANYING NEW APPLICATION.")	$
[ ]	Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached ($130.00; 37 C.F.R. Sections 1.47 and 1.17(I))	$
[ ]	For processing an application with a specification in a non-English language ($130.00; 37 C.F.R. Sections 1.52(d) and 1.17(k))	$
[ ]	Processing and retention fee ($130.00; 37 C.F.R. Sections 1.53(d) and 1.21(l))	$
[ ]	Fee for international-type search report ($40.00; 37 C.F.R. Section 1.21(e))	$

NOTE: 37 C.F.R. Section 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 C.F.R. Section 1.53(f) and this, as well as the changes to 37 C.F.R. Section 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of Section 1.21(l) must be paid, within 1 year from notification under Section 53(f).

Total Fees Enclosed $ 690.00

14. Method of Payment of Fees

[X] Check in the amount of $____690.00__.

[ ] Charge Account No. _____ in the amount of $_____.
A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 C.F.R. Section 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: *If no fees are to be paid on filing, the following items should not be completed.*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.*

[X] The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. 12-0425.

[X] 37 C.F.R. Section 1.16(a), (f) or (g) (filing fees)

[ ] 37 C.F.R. Section 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. Section 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

[ ] 37 C.F.R. Section 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

[ ] 37 C.F.R. Section 1.17(a)(1)-(5) (extension fees pursuant to Section 1.136(a).

[X] 37 C.F.R. Section 1.17 (application processing fees)

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under Section 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in Section 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. Section 1.136(a)(3).

[ ] 37 C.F.R. Section 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. Section 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. Section 1.311(b)).

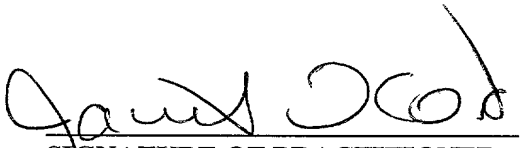
NOTE: 37 C.F.R. Section 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . issue fee." From the wording of 37 C.F.R. Section 1.28(b), (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions as to Overpayment

NOTE: ". . . Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. Section 1.26(a).

☒ Credit Account No. 12-0425.

[ ] Refund



SIGNATURE OF PRACTITIONER

Reg. No.33,778

JANET I. CORD
(type or print name of practitioner)

Tel. No.: (212)708-1935

LADAS & PARRY
P.O. Address

Customer No.:

26 WEST 61ST STREET
NEW YORK, N.Y. 10023

[X] **Incorporation by reference of added pages**

(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

[X] Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added 5

[ ] Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added _____

[ ] Plus added pages deleting names of inventor(s) named on prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.

Number of pages added _____

[ ] Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added _____

[ ] **Statement Where No Further Pages Added**

(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)

[ ] This transmittal ends with this page.

ADDED PAGE(S) FOR SPECIAL COMMENTS FOR NEW APPLICATION TRANSMITTAL

Added page _____

(Added Page(s) for Special Comments for New Application Transmittal)

Practitioner's Docket No. U 012799-1 ***PATENT***

ADDED PAGES FOR APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED

NOTE: See 37 CFR 1.78.

17. Relate Back

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

(complete the following, if applicable)

[X] Amend the specification by inserting, before the first line, the following sentence:

A. 35 U.S.C. 119(e)

NOTE: "Any nonprovisional application claiming the benefit of one or more prior filed copending provisional applications must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior provisional application, identifying it as a provisional application, and including the provisional application number (consisting of series code and serial number)." 37 C.F.R. § 1.78(a)(4).

[ ] "This application claims the benefit of U.S. Provisional Application(s) No(s).:

APPLICATION NO(S).:	**FILING DATE**
_____/_____	_____
_____/_____	_____
_____/_____	_____

and incorporates the same by reference."

B. 35 U.S.C. 120, 121 and 365(c)

NOTE: "Except for a continued prosecution application filed under § 1.53(d), any nonprovisional application claiming the benefit of one or more prior filed copending nonprovisional applications or international applications designating the United States of America must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior application, identifying it by application number (consisting of the series code and serial number) or international application number and international filing date and indicating the relationship of the applications. . . . Cross-references to other related applications may be made when appropriate." (See § 1.14(a)). 37 C.F.R. § 1.78(a)(2).

[X] "This application is a

[ ] continuation

[X] continuation-in-part

[ ] divisional

of copending application(s)

[X] application number 09/248,382 filed on FEBRUARY 11, 1999 and application number 09/248,381 filed FEBRUARY 11, 1999 which is a CIP of application number 08/727,679 filed OCTOBER 8, 1996.

[ ] which is

[ ] International Application _____ filed on _____ and which designated the U.S.,

claims the benefit thereof and incorporates the same by reference."

NOTE: The proper reference to a prior filed PCT application that entered the U.S. national phase is the U.S. serial number and the filing date of the PCT application that designated the U.S.

NOTE: (1) Where the application being transmitted adds subject matter to the International Application, then the filing can be as a continuation-in-part or (2) if it is desired to do so for other reasons then the filing can be as a continuation.

NOTE: The deadline for entering the national phase in the U.S. for an international application was clarified in the Notice of April 28, 1987 (1079 O.G. 32 to 46) as follows:

"The Patent and Trademark Office considers the International application to be pending until the 22nd month from the priority date if the United States has been designated and no Demand for International Preliminary Examination has been filed prior to the expiration of the 19th month from the priority date and until the 32nd month from the priority date if a Demand for International Preliminary Examination which elected the United States of America has been filed prior to the expiration of the 19th month from the priority date, provided that a copy of the international application has been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively. If a copy of the international application has not been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively, the international application becomes abandoned as to the United States 20 or 30 months from the priority date respectively. These periods have been placed in the rules as paragraph (h) of § 1.494 and paragraph (i) of § 1.495. A continuing application under 35 U.S.C. 365(c) and 120 may be filed anytime during the pendency of the international application."

[ ] "The nonprovisional application designated above, namely application _____/_____, filed _____, claims the benefit of U.S. Provisional Application(s) No(s).:

APPLICATION NO(S).:	**FILING DATE**
_____/_____	_____"
_____/_____	_____"
_____/_____	_____"

[ ] Where more than one reference is made above please combine all references into one sentence.

18. Relate Back—35 U.S.C. 119 Priority Claim for Prior Application

The prior U.S. application(s), including any prior International Application designating the U.S., identified above in item 17B, in turn itself claim(s) foreign priority(ies) as follows:

Country	Appln. no.	Filed
INDIA	343/DEL/98	FEBRUARY 11, 1998
INDIA	342/DEL/98	FEBRUARY 11, 1998

The certified copy(ies) has (have)

[ ] been filed on _____, in prior application _____, which was filed on _____.

[ ] is (are) attached.

WARNING: *The certified copy of the priority application that may have been communicated to the PTO by the International Bureau may not be relied on without any need to file a certified copy of the priority application in the continuing application. This is so because the certified copy of the priority application communicated by the International Bureau is placed in a folder and is not assigned a U.S. serial number unless the national stage is entered. Such folders are disposed of if the national stage is not entered. Therefore, such certified copies may not be available if needed later in the prosecution of a continuing application. An alternative would be to physically remove the priority documents from the folders and transfer them to the continuing application. The resources required to request transfer, retrieve the folders, make suitable record notations, transfer the certified copies, enter and make a record of such copies in the Continuing Application are substantial. Accordingly, the priority documents in folders of international applications that have not entered the national stage may not be relied on. Notice of April 28, 1987 (1079 O.G. 32 to 46).*

19. Maintenance of Copendency of Prior Application

NOTE: The PTO finds it useful if a copy of the petition filed in the prior application extending the term for response is filed with the papers constituting the filing of the continuation application. Notice of November 5, 1985 (1060 O.G. 27).

A. [ ] Extension of time in prior application

*(This item **must** be completed and the papers filed **in the prior application,** if the period set in the prior application has run.)*

[ ] A petition and fee extends the term in the pending **prior** application until _____.

[ ] A **copy** of the petition filed in prior application is attached.

B. [ ] Conditional Petition for Extension of Time in Prior Application

[ ] A conditional petition for extension of time is being filed in the pending **prior** application.

[ ] **A copy** of the conditional petition filed in the prior application is attached.

C. [ ] No extension is necessary in Prior Application
[ ] Issue Fee paid _____

20. Further Inventorship Statement Where Benefit of Prior Application(s) Claimed

(complete applicable item (a), (b) and/or (c) below)

(a) [ ] This application discloses and claims only subject matter disclosed in the prior application whose particulars are set out above and the inventor(s) in this application are

[ ] the same.

[ ] less than those named in the prior application. It is requested that the following inventor(s) identified for the prior application be deleted:

_____

(type name(s) of inventor(s) to be deleted)

(b) [ ] This application discloses and claims additional disclosure by amendment and a new declaration or oath is being filed. With respect to the prior application, the inventor(s) in this application are

[ ] the same.

[ ] the following additional inventor(s) have been added:

_____

(type name(s) of inventor(s) to be deleted)

(c) [ ] The inventorship for all the claims in this application are

[ ] the same.

[ ] not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made

[ ] is submitted.

[ ] will be submitted.

21. Abandonment of Prior Application *(if applicable)*

[ ] Please abandon the prior application at a time while the prior application is pending, or when the petition for extension of time or to revive in that application is granted, and when this application is granted a filing date, so as to make this application copending with said prior application.

NOTE: According to the Notice of May 13, 1983 (103, TMOG 6-7), the filing of a continuation or continuation-in- part application is a proper response with respect to a petition for extension of time or a petition to revive and should include the express abandonment of the prior application conditioned upon the granting of the petition and the granting of a filing date to the continuing application.

22. Petition for Suspension of Prosecution for the Time Necessary to File an Amendment

WARNING: *"The claims of a new application may be finally rejected in the first Office action in those situations where (1) the new application is a continuing application of, or a substitute for, an earlier application, and (2) all the claims of the new application (a) are drawn to the same invention claimed in the earlier application, and (b) would have been properly finally rejected on the grounds of art of record in the next Office action if they had been entered in the earlier application." MPEP, § 706.07(b).*

NOTE: Where it is possible that the claims on file will give rise to a first action final for this continuation application and for some reason an amendment cannot be filed promptly (e.g., experimental data is being gathered) it may be desirable to file a petition for suspension of prosecution for the time necessary.

(check the next item, if applicable)

[ ] There is provided herewith a Petition To Suspend Prosecution for the Time Necessary to File An Amendment (New Application Filed Concurrently)

23. Small Entity (37 CFR § 1.28(a))

[ ] Applicant has established small entity status by the filing of a statement in parent application _____ on _____.

[ ] A copy of the statement previously filed is included.

WARNING: *See 37 CFR § 1.28(a).*

24. NOTIFICATION IN PARENT APPLICATION OF THIS FILING

[ ] A notification of the filing of this
(check one of the following)

[ ] continuation

[ ] continuation-in-part

[ ] divisional

is being filed in the parent application, from which this application claims priority under 35 U.S.C. § 120.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: ANAND C. **BURMAN**, et al

For: BOMBESIN ANALOGS FOR TREATMENT OF CANCER

Attorney Docket No.: U 012799-1

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Please insert the attached sequence listing after page 21 of the specification.

Respectfully submitted,

JANET I. CORD
LADAS & PARRY
26 WEST 61ST STREET
NEW YORK, NEW YORK 10023
REG.NO.33778(212)708-1935

CERTIFICATE UNDER 37 CFR 1.10

I hereby certify that this paper is being deposited with the United States Postal Service on this date JULY 31, 2000 in an envelope as "EXPRESS MAIL POST OFFICE TO ADDRESSEE" Mailing Label Number EL386270297US addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231

GERALDINE MARTI
(Type or print name of person mailing paper)

(Signature of person mailing paper)

NOTE: Each paper or fee referred to as enclosed herein has the number of the "EXPRESS MAIL" mailing label place thereon prior to mailing 37 CFR 1.16(b).

BOMBESIN ANALOGS FOR TREATMENT OF CANCER

FIELD OF INVENTION

The present invention encompasses novel peptides that are antagonists to bombesin and bombesin like peptides and are useful in the treatment of cancer. The invention particularly relates to the design and synthesis of the novel peptides incorporating α,α-amino acids in a site specific manner. The invention encompasses methods for the generation of these peptides, compositions containing the peptides and the pharmacological applications of these peptides especially in the treatment and prevention of cancer.

BACKGROUND OF THE INVENTION

Bombesin is a 14 amino acid peptide which was first isolated from the skin of the frog Bombina bombina (Anastasi et al., Experientia, 1971, 27, 166) and has the sequence:

pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-
Leu-Met-NH_2 (SEQ ID NO: 1)

Gastrin releasing peptide (GRP) is a 27 amino acid peptide isolated from the porcine gut. The last ten amino acids at the C-terminus of gastrin releasing peptide correspond with one amino acid alteration (3) to the last ten amino acids of bombesin, viz:

H-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
(SEQ ID NO:2).

It has been reported (J. H. Walsh and J. R. Reeve, Peptides 6, (3), 63-68, (1985)) that bombesin and bombesin-like peptides such as gastrin releasing peptide (GRP) are secreted by human small-cell lung cancer (SCLC) cells. It has been postulated (P. J. Woll and E. Rozengurt, PNAS 85, 1859-1863, (1988)) that gastrin releasing factor antagonists would bind competitively to bombesin receptors in animals and would therefore be of use in the treatment of SCLC and/or in the control of clinical symptoms associated with this disease and due to hypersecretion of this peptide hormone. Analogues of bombesin / GRP have been shown to inhibit the binding of gastrin releasing peptide to a SCLC cell line and to inhibit the growth of SCLC cells in-vitro and in-vivo (S. Mahmoud et al., Cancer Research, 1991, 51, 1798; Moody TW et al., Life Sci., 1995, 56, 521; Moody TW et al.,

Peptides, 1996, 17, 1337). After Bombesin/GRP cell receptors were established on SCLC cells, receptors were also found to be present on human prostate cells. Reile H et al., (Prostate, 1994, 25: 29-38) showed that the PC-3 and DU-145 human prostate cancer cell lines possess specific high-affinity receptors for bombesin/GRP and are suitable models for the evaluation of anti-neoplastic activity of new bombesin/GRP antagonists in the treatment of androgen-dependent prostate cancer. Bombesin also increased the penetration of the two human prostatic carcinoma cell lines, the relatively indolent LNCaP cells and the aggressively growing and invasive PC-3 cells, in an in vitro invasion of reconstituted basement membrane (Matrigel) (Hoosein NM et al., J Urol, 149(5): 1209-1213). High-affinity binding sites for GRP were found on human colorectal cancer tissue (Preston, SR. et al, Br. J. Can., 1995, 71, 1087), suggesting that bombesin-like peptides may have a role in the pathogenesis of colorectal cancer, and bombesin receptor antagonists may be of value in the treatment of receptor-positive tumours. Inhibitory effects of bombesin/GRP antagonist RC-3095 and somatostatin analogue RC-160 were also seen on growth of HT-29 human colon cancer xenografts in nude mice (Radulovic S et al., Acta Oncol, 1994, 33(6): 693-701).

Studies with the anti-bombesin/GRP antibodies lead to the hypothesis that it may be possible to disrupt the autocrine growth cycle of bombesin/GRP using designed peptide receptor antagonists. Since then several types of Bombesin antagonists have been reported. These antagonists have been defined by type and position of the substitutions of the natural sequence. Early receptor antagonists suffered from low potency, lack of specificity, and toxicity, which presented serious problems with their scientific and therapeutic use.

More recent work has concentrated on modification of the carboxy terminal (C-terminal) region of these peptides to interrupt the receptor interaction utilizing a variety of different types of C-terminal modified analogs. These have included incorporation of D-amino acids, non-peptide bonds for example (psi. CH_2NH), amide, and ester modifications. These alterations gave rise to certain peptides having improved characteristics (Staley J et al., Peptides, 1991, 12(1): 145-9; Coy DH et al., J Natl Cancer Inst Monogr, 1992, 13: 133-9). Other patents that describes bombesin and related analogs are:

USP5,834,433 (1998)

USP 5,723,578 (1998)

USP 5,620,959 (1997)

USP 5,620,955 (1997)

USP 5,428,019 (1995)

USP 5,369,094 (1994)

USP 5,084,555 (1992)

A Bombesin/GRP antagonist (RC-3940-II) was found to inhibit the proliferation of SW-1990 human pancreatic adenocarcinoma cells in vivo and in vitro (Qin, Y. et al., 1995, Int. J. Cancer, 63, 257). Similar effect was seen with bombesin/GRP antagonist RC-3095 on the growth of CFPAC-1 human pancreatic cancer cells transplanted to nude mice or cultured in vitro (Qin Y et al., Can Res, 1994, 54(4): 1035-41).

As reported earlier, the autocrine growth cycle of bombesin/GRP in SCLC can be disrupted by bombesin/GRP antagonists such as [Psi 13,14] bombesin. Several bombesin analogues were solid phase synthesized and incubated with intact SCLC cells at 37°C in RPMI medium in a time course fashion (0-1080 minutes) to determine enzymatic stability. The proteolytic stability of the compounds was determined by subsequent HPLC analysis. [Psi 13, 14] Bombesin was found to be very stable to metabolic enzymes (T1/2= 646 min.) and inhibited SCLC xenograft formation in vivo in a dose-dependent manner (Davis TP et al., Peptides, 1992, 13(2): 401-7).

Female athymic nude mice bearing xenografts of the MCF-7 MIII human breast cancer cell line were treated for 7 weeks with bombesin/GRP antagonist (DTpi6, Leul3 psi[CH_2NH]-Leu14) bombesin (6-14)(RC-3095) injected subcutaneously daily at a dose of 20 μg and LHRH antagonist SB-75 (Cetrorelix) administered biweekly in the form of microgranules releasing 45 μg/ day. After 2 weeks of treatment, a significant inhibition of tumor volume was observed in the groups treated with RC-3095 alone or in combination with SB-75 (Yano T et al., Cancer, 1994, 73(4): 1229-38).

Pinski J et al., (Int. J. Cancer, 1994, 57(4): 574-580), demonstrated for the first time that the growth of gastrin-responsive human gastric carcinoma

MKN45 cell line xenografts in nude mice could be inhibited not only by somatostatin analogues, but also by administration of modern bombesin/GRP antagonists, such as RC-3095, or a combination of these. RC-3095 also effectively inhibited tumor growth in nude mice bearing xenografts of the human gastric cancer cell line Hs746T (Qin Y et al., J Cancer Res Clin Oncol, 1994,120(9):519-528).

This invention describes the preparation and use of peptide analogs of bombesin/GRP using constrained amino acids and their use for cancer therapy, alone, or in combination or as an adjunct to or with other chemotherapeutic agents and compounds.

The design of conformationally constrained bioactive peptide derivatives has been one of the widely used approaches for the development of peptide-based therapeutic agents. Non-standard amino acids with strong conformational preferences may be used to direct the course of polypeptide chain folding, by imposing local stereochemical constraints, in de novo approaches to peptide design. The conformational characteristics of α,α-dialkylated amino acids have been well studied. The incorporation of these amino acids restricts the rotation of ϕ, Ψ angles, within the molecule, thereby stabilizing a desired peptide conformation. The prototypic member of α,α-dialkylated aminoacids, α-aminoisobutyric acid (Aib) or α,α-dimethylglycine has been shown to induce (β-turn or helical conformation when incorporated in a peptide sequence (Prasad and Balaram, (1984); CRC Crit. Rev. Biochem. 16, 307-347; Karle and Balaram (1990) Biochemistry 29, 6747-6756). The conformational properties of the higher homologs of α,α-dialkylated amino acids such as diethylglycine (Deg), di-n-propylglycine (Dpg) and di-n-butylglycine (Dbg) as well as the cyclic side chain analogs of α,α-dialkylated amino acids such as 1-aminocyclopentane carboxylic acid (Ac5c), 1-aminocyclohexane carboxylic acid (Ac6c), 1-aminocycloheptane carboxylic acid (Ac7c) and 1-aminocyclooctane carboxylic acid (Ac8c) have also been shown to induce folded conformation (Prasad et al., (1995), Biopolymers 35, 11-20; Karle et al., (1995); J. Amer. Chem. Soc. 117, 9632-9637). α,α-dialkylated amino acids have been used in the design of highly potent chemotactic peptide analogs (Prasad et al., (1996) Int. J. Peptide Proteins RCS. 48, 312-318).

The present invention exploits the conformational properties of α,α-

dialkylated amino acids for the design of biologically active peptide derivatives, taking bombesin as the model system under consideration. Furthermore, it has been shown that lipophilization of bioactive peptides improves their stability, bioavailability and the ability to permeate biomembranes (Dasgupta, P et al; 1999, Pharmaceutical Res. 16, 1047-1053; Gozes, I, et al 1996, Proc. Natl. Acad. Sci. USA, 93, 427-432). In the present invention, we have also synthesized peptide derivatives having N-terminal alkanoyl groups from C2-C16 carbon atoms, which retain anticancer activity.

The present invention exploits the conformational properties of α,α-dialkylated amino acids for the design of biologically active peptide derivatives, taking bombesin as the model system under consideration. Furthermore, it has been shown that lipophilization of bioactive peptides improves their stability, bioavailability and the ability to permeate biomembranes (Dasgupta, P et al; 1999, Pharmaceutical Res. 16, 1047-1053; Goes, L, et al., 1996, Proc. Natl. Acad. Sci. USA, 93, 427-432).

Throughout the specification and claims the amino acid residues are designated by their standard abbreviations. Amino acids denote L-configuration unless otherwise indicated by D or DL appearing before the symbol and separated from it by a hypen. Throughout the specification and claims, the following abbreviations are used with the following meanings:

BOP: Benzotriazole-l-yl-oxy-tris-(dimethylamino)-phosphonium hexfluorophosphate

PyBOP: Benzotriazole-l-yl-oxy-tris-pyrrolidino-phosphonium hexofluorophospate

TBTU: 2-(1H-Benzotriazole-lyl)-1,1,3,3-tetramethyluronium tetrafluroborate

HBTU: O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexofluoro-phosphate

HOBt: 1-Hydroxy Benzotriazole

DCC: Dicyclohexyl carbodiimide

DIPCDI: Diisopropyl carbodiimide

DIEA: Diisopropyl ethylamine

DMF: Dimethyl formamide

DCM: Dichloromethane

NMP: N-Methyl-2-pyrrolidinone

TFA: trifluoroacetic acid

SUMMARY OF INVENTION

The present invention provides novel polypeptides of the following general formula,

X-D-Phe-Gln-R1-R2-Val-R3-His-R4-NH_2

wherein X is acetyl or straight, branched, or cyclic alkanoyl group from 3-16 carbon atoms, or X is deleted,

R1 is Trp or D-Trp,

R2 is Ala, Aib or Deg,

R3 is Gly, Aib, Deg, Dpg or Ac5c,

R4 is Leu or Ile or a hydrolyzable carboxy protecting group;

or a pharmaceutically acceptable salt of the polypeptide. At least one of R2 or R3 is a non-standard amino acid. The invention also encompasses methods for making the peptides, compositions containing the peptides and use of the peptides.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel polypeptides of the following general formula,

X-D-Phe-Gln-R1-R2-Val-R3-His-R4-NH_2

wherein X is acetyl or straight, branched, or cyclic alkanoyl group from 3-16 carbon atoms, or X is deleted,

R1 is Trp or D-Trp,

R2 is Ala, Aib or Deg,

R3 is Gly, Aib, Deg, Dpg or Ac5c,

R4 is Leu or Ile or a hydrolyzable carboxy protecting group;

or a pharmaceutically acceptable salt of the polypeptide. At least one of R2 or R3 is a non-standard amino acid.

A hydrolyzable carboxy protecting group are those groups which on hydrolysis converts to carboxylic group such as -$COONH_2$, -COOMe, etc.

The preferred alkanoyl groups are acetyl, n-butanoyl, n-hexanoyl, n-

octanoyl, lauroyl, myristoyl, palmitoyl, isohexanoyl, cyclohexanoyl, cyclopentyl-carbonyl, n-heptanoyl, n-decanoyl, n-undecanoyl and 3,7-dimethyloctanoyl.

Salts encompassed within the term "pharmaceutically acceptable salts" refer to non-toxic salts of the compounds of this invention. Representative salts and esters include:

acetate, ascorbate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, camsylate, carbonate, citrate, dihydrochloride, methanesulfonate, ethanesulfonate, p-toluenesulfonate, cyclohexylsulfamate, quinate, edetate, edisylate, estolate, esylate, fumaxate, gluconate, glutamate, glycerophophates, hydrobromide, hydrochloride, hydroxynaphthoate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, mucate, napsylate, nitrate, n-methylglucamine, oleate, oxalate, palmoates, pamoate (embonate), palmitate, pantothenate, perchlorates, phosphate/diphosphate, polygalacturonate, salicylates, stearate, succinates, sulfate, sulfamate, subacetate, succinate, tannate, tartrate, trifluoroacetate, tosylate and valerate.

Other salts include Ca, Li, Mg, Na and K salts; salts of amino acids such lysine or arginine; guanidine, diethanolamine or choline; ammonium, substituted ammonium salts or aluminum salts.

The salts can be prepared by standard techniques.

Preferred peptides of this invention are:

D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO:3)

D-Phe-Gln-Trp-Aib-Val-Gly -His-Leu-NH_2 (SEQ ID NO:4)

D-Phe-Gln-D-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO:5)

D-Phe-Gln-Trp-Aib-Val-Gly-His-Ile-NH_2 (SEQ ID NO:6)

D-Phe-Gln-Trp-Ala-Val-Aib-His-Ile-NH_2 (SEQ ID NO:7)

D-Phe-Gln-D-Trp-Ala-Val-Dpg-His-Leu-NH_2 (SEQ ID NO:8)

D-Phe-Gln-Trp-Deg-Val-Gly-His-Leu-NH_2 (SEQ ID NO:9)

D-Phe-Gln-Trp-Ala-Val-Ac5c-His-Leu-NH_2 (SEQ ID NO: 10)

Butanoyl-D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO: 11)

Octanoyl-D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO: 12)

The present invention also envisages methods of prevention and treatment of cancer using the polypeptides of the present invention, pharmaceutical compositions comprising such polypeptides and processes for their preparation. These peptides possess antagonist properties against bombesin and bombesin-like peptides and are useful in the prevention and treatment of malignant diseases.

Suitable routes for administration of the peptides are those known in the art and include oral, rectal, transdermal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intradedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Pharmaceutical compositions suitable for use in present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers, excipients, diluents, solvents, flavorings, colorants etc. The preparations may be formulated in any form including but not limited to tablets, dragees, capsules, powders, syrups, suspensions, slurries, time released formulations, sustained release formulations, pills, granules, emulsions, patches, injections, solutions, liposomes or nanoparticles.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition.

The term "an effective amount" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal or human that is being sought.

Toxicity and therapeutic efficacy of the peptides of this invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals.

The novel peptide analogs embodied in the present invention contain amino acids, namely α,α-dialkylated amino acids, which have been known to induce highly specific constraints in the peptide backbone. The α,α-dialkylated amino acids, used in the present invention are synthesized from the corresponding ketones. In a preferred embodiment of the invention, the ketones are first converted

into the corresponding hydantoins which are hydrolyzed using a strong acid or base, preferably H_2SO_4, HCl, NaOH or Na_2CO_3 to yield the aforesaid amino acids. In a preferred embodiment of the present invention, 60% sulphuric acid has been employed as the hydrolyzing agent.

The present invention also provides a solid phase synthesis process for the preparation of peptide analogs of the general formula (I):

X-D-Phe-Gln-R1-R2-Val-R3-His-R4-NH_2

wherein X is acetyl or straight, branched, or cyclic alkanoyl group from 3-16 carbon atoms or X is deleted,

R1 is Trp or D-Trp,

R2 is Ala, Aib or Deg,

R3 is Gly, Aib, Deg, Dpg or Ac5c,

R4 is Leu or Ile

which comprises sequentially loading the corresponding protected α,α-dialkylated amino acids in sequential cycles to the amino terminus of a solid phase resin, coupling the amino acids in the presence of conventional solvents and reagents to assemble a peptide-resin assembly, removing the protecting groups and cleaving the peptide from the resin to obtain a crude peptide analog.

The novel peptides in the present invention have been generated by using solid phase techniques or by a combination of solution phase procedures and solid phase techniques or by fragment condensation. These methods for the chemical synthesis of polypeptides are well known in the art (Stewart and Young, 1969, Solid Phase Peptide Synthesis, W.H. Freeman & Co.).

In a preferred embodiment of the present invention the peptides were synthesized using the Fmoc strategy, on a semi automatic peptide synthesizer (CS Bio, Model 536), using optimum side chain protection. The peptides were assembled from C-terminus to N-terminus. Peptides amidated at the carboxy-terminus were synthesized using the Rink Amide resin. The loading of the first Fmoc protected amino acid was achieved via an amide bond formation with the solid support, mediated by Diiopropylcarbodiimide (DIPCDI) and HOBt. Substitution levels for automated synthesis were preferably between 0.2 and 0.6 mmole amino acid per gram resin.

The resin employed for the synthesis of carboxy-terminal amidated peptide analogs was 4-(2', 4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxymethyl derivatized polystyrene 1% divinylbenzene (Rink Amide) resin (100-200 mesh), procured from Calbioichem-Novabiochem Corp., La Jolla, U.S.A., (0.47 milliequivalent NH_2/g resin).

The N-terminal amino group was protected by 9-fluorenylmethoxy-carbonyl (Fmoc) group. Trityl (trt) or t-butyloxycarbonyl (Boc) were the preferred protecting groups for imadazole group of Histidine residue. The hydroxyl groups of Serine, Threonine and Tyrosine were preferably protected by t-butyl group (tBu) 2,2,5,7,8-pentamethyl-chroman-6-sulfonyl (Pmc) or 2,2,4,7,-pentamethyl-dihydro-benzenofuran-5 5-sulfonyl (Pbf) were the preferred protecting groups for the guandino group of Arginine. Trityl was the preferred protecting group for Asparagine and Glutamine and tertiary butyl group (tBu) was the preferred protecting group for Aspartic acid and Glutamic acid. The tryptophan residue was either left unprotected or used with Boc protection. The side chain amino group of Lysine was protected using Boc group preferably.

In a preferred embodiment of the invention, 2-8 equivalents of Fmoc protected amino acid per resin nitrogen equivalent were used. The activating reagents used for coupling amino acids to the resin, in solid phase peptide synthesis, are well known in the art. These include DCC, DIPCDI, DIEA, BOP, PyBOP, HBTU, TBTU, or HOBt. Preferably, DCC, DIPCDI/HOBt or HBTU/HOBT and DIEA were used as activating reagents in the coupling reactions.

The protected amino acids were either activated in situ or added in the form of preactivated esters known in the art such as NHS esters, Opfp esters etc. Atherton, E. et. al, 1988, J. Chem. Soc., Perkin Trans.I, 2887; Bodansky, M. in "The Peptides, Analysis, Synthesis and Biology (E. Gross, J, Meienhofer, eds) Vol. I, Academic Press, New York, 1979, 106.

The coupling reaction was carried out in DMF, DCM or NMP or a mixture of these solvents and was monitored by Kaiser test (Kaiser et al., Anal. Biochem., 34, 595-598 (1970)). In case of a positive Kaiser test, the appropriate amino acid was re-coupled using freshly prepared activated reagents.

After the assembly of the peptide was completed, the amino-terminal

Fmoc group was removed and then the peptide-resin was washed with methanol and dried. The peptides were then deprotected and cleaved from the resin support by treatment with trifluoroacetic acid, crystalline phenol, ethanedithiol, thioanisole and de-ionized water for 1.5 to 5 hours at room temperature. The crude peptide was obtained by precipitation with cold dry ether, filtered, dissolved, and lyophilized.

The resulting crude peptide was purified by preperative high performance liquid chromatography (HPLC) using a LiChroCART® C,8 (250. Times. 10) reverse phase column (Merck, Darmstadt, Germany) on a Preparative HPLC system (Shimadzu Corporation, Japan) using a gradient of 0.1 % TFA in acetonitrile and water. The eluted fractions were reanalyzed on Analytical HPLC system (Shimadzu Corporation, Japan) using a C18 LiChrospherg®, WP-300 (300 X 4) reverse- phase column. Acetonitrile was evaporated and the fractions were lyophilized to obtain the pure peptide. The identity of each peptide was confirmed by electron-spray mass spectroscopy.

Synthesis Of Peptides

A peptide of the present invention can be made by exclusively solid phase techniques, by partial solid phase/solution phase techniques and/or fragment condensation. Preferred, semi-automated, stepwise solid phase methods for synthesis of peptides of the invention are provided in the examples discussed in the subsequent section of this document.

The present invention will be further described in detail with reference to the following examples, as will be appreciated by a person skilled in the art are merely illustrative and should not be construed as limiting. Various other modifications of the invention will be possible without departing from the spirit and scope of the present invention.

EXAMPLE 1

First loading on Rink Amide Resin

A typical preparation of the Fmoc-Leu-Rink Amide Resin was carried out using 0.5g of 4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)phenoxymethyl derivatized polystyrene 1% divinylbenzene (Rink Amide) resin (0.7 mM/g) (100-200 mesh), procured from Advanced Chemtech, Louisville, KY, U.S.A., (0.7 milliequivalent NH_2 resin). Swelling of the resin was typically carried out in

dichloromethane measuring to volumes 10-40 ml/g resin. The resin was allowed to swell in methylene chloride (2 X 25 ml, for 10 min.). It was washed once in dimethylformamide (DMF) for 1 min. All solvents in the protocol were added in 20 ml portions per cycle. The Fmoc-protecting group on the resin was removed by following steps 3-7 in the protocol. The deprotection of the Fmoc group was checked by the presence of blue beads in Kaiser test. For loading of the first amino acid on the free amino (NH_2) group of the resin, the first amino acid, Fmoc-Leu-OH, was weighed in three to six fold excess, along with a similar fold excess of HOBt, in the amino acid vessel of the peptide synthesizer. These were dissolved in dimethylformamide (A.C.S. grade) (J.T.Baker, Phillipsburg, New Jersey, U.S.A.) and activated with DIPCDI, just prior to the addition to the resin in the reaction vessel of the peptide synthesizer. HOBt was added in all coupling reactions, especially in the case of Gln and His. The coupling reaction was carried out for a period ranging from 1-3 hours. The loading of the amino acid on the resin was confirmed by the presence of colorless beads in the Kaiser Test. The loading efficiency was ascertained by the increase of weight of the resin after the addition of the amino acid.

EXAMPLE 2

Synthesis of D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO: 3)

The synthesis of SEQ ID NO: 3, amidated at the carboxy- terminus, was initiated by using all of the resin loaded with Fmoc-Leu-OH as prepared in Example 1 above. This was subjected to stepwise deprotection and coupling steps as in steps 1-10 of the synthesis cycle. In each coupling reaction, a two to six fold excess of amino acid, DIPCDI and HOBt were used. Upon completion of synthesis and removal of the N-terminal Fmoc protecting group (steps 1-6 of the synthesis cycle), the peptide- resin was washed twice with methanol, dried and weighed to obtain 0.649g. This was subjected to cleavage in a cleavage mixture consisting of trifluoroacetic acid and scavengers, ethanedithol, crystalline phenol and thioanisole and water for a period of 1.5 to 5 hours at room temperature with continuous stirring. The peptide was precipitated using cold dry ether to obtain ~ 330 mg of crude peptide. The crude peptide was purified on a C18 preperative reverse phase HPLC column (250 X 10) on a gradient system comprising acetonitrile and water in

0.1 % TFA as described previously in the art. The prominent peaks were collected and lyophilized, reanalyzed on analytical HPLC and subjected to mass spectrometry. There was a good agreement between the observed molecular weight and calculated molecular weight (Calculated Mass ~ 983; Observed Mass ~ 984.2). The pure peptide was then used for bioassays.

EXAMPLE 3

Synthesis of D-Phe-Gln-Trp-Aib-Val-Gly-His-Leu-NH_2 (SEQ ID NO:4)

The synthesis, cleavage and lyophilization steps were carried out as in the Example 2 above using the appropriate amino acids. The calculated mass was ~ 969 and the observed mass was 970.4.

EXAMPLE 4

Synthesis of D-Phe-Gln-D-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO:5)

The synthesis, cleavage and lyophilization steps were carried out as in the Example 2 above using the appropriate amino acids. The calculated mass was ~ 983 and the observed mass was 984.30.

EXAMPLE 5

Synthesis of D-Phe-Gln-Trp-Aib-Val-Gly-His-Ile-NH_2 (SEQ ID NO:6)

The synthesis, cleavage and lyophilization steps were carried out as in the Example 2 above using the appropriate amino acids. The calculated mass was ~ 969 and the observed mass was 970.2.

EXAMPLE 6

Synthesis of D-Phe-Gln-Trp-Ala-Val-Aib-His-Ile-NH_2 (SEQ ID NO:7)

The synthesis, cleavage and lyophilization steps were carried out as in the Example 2 above using the appropriate amino acids. The calculated mass was ~ 983 and the observed mass was 984.2.

EXAMPLE 7

Synthesis of D-Phe-Gln-D-Trp-Ala-Val-Dpg -His-Leu-NH_2 (SEQ ID NO:8)

The synthesis, cleavage and lyophilization steps were carried out as in the Example 2 above using the appropriate amino acids. The calculated mass was ~ 1039 and the 25 observed mass was 1040.4.

EXAMPLE 8

Synthesis D-Phe-Gln-Trp-Deg-Val-Gly-His-Leu-NH_2 (SEQ ID NO:9)

The synthesis, cleavage and lyophilization steps were carried out as in the Example 2 above using the appropriate amino acids. The calculated mass was ~ 997 and the observed mass was 998.5.

EXAMPLE 9

Synthesis of D-Phe-Gln-Trp-Ala-Val-Ac5c-His-Leu-NH_2 (SEQ ID NO: 10)

The synthesis, cleavage and lyophilization steps were carried out as in the Example 2 above using the appropriate amino acids. The calculated mass was ~ 1009 and the observed mass was 1010.4.

EXAMPLE 10

Synthesis of Butanoyl-D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO: 11)

The conjugation of the butanoyl group at the N-terminal position was done on solid phase. The above peptide sequence was synthesized on resin as described in Example 2. After the deprotection of D-Arg amino acid it was further coupled with butanoic acid in DMF using DIPCDI and HOBT. The cleavage and purification was further carried out following the standard protocol as described in Example 2. The final peptide was further analyzed by mass spectroscopy. The calculated mass and observed were in good agreement. (calculated mass = ~ 1053, observed mass = 1054.2).

EXAMPLE 11

Synthesis of Octanoyl-D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO: 12)

The conjugation of the octanoyl group at the N-terminal position after the peptide synthesized as described in Example 2 was done on solid phase using octanoic acid in DMF using DIPCDI and HOBT. The cleavage and purification was further carried out following the standard protocol as described in Example 2. The final purified peptide was further analyzed by mass spectroscopy. The calculated mass and observed were in good agreement. (calculated mass = ~ 1109, observed mass = 1110.5).

BIOLOGICAL ACTIVITY OF PEPTIDES

The cytoxicity of the peptide analog was carried out by two day MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. MTT assay

is based on the principle of uptake of MTT, a tetrazolium salt, by metabolically active cells where it is metabolized by active mitochondria into a blue colored formazon product, which can be read spectrometrically (J. of Immunological Methods 65: 55-63, 1983). To prepare the MTT stock solution needed, MTT was dissolved in phosphate buffered saline with a pH of 7.4 to obtain an MTT concentration of 5 mg/ml; the resulting mixture was filtered through a 0.22 micron filter to sterilize and remove a small amount of insoluble residue. This filtered mixture was the MTT stock solution.

Briefly, for each tumor type, 10,000 cells were seeded in 96-well tissue culture plate and incubated with each peptide concentration individually in a CO_2 incubator for 48 hrs. The peptide analog at different concentrations was added once every 24 hrs during the incubation period. Control cultures, which were not treated with the peptide was similarly incubated. The assay was terminated by adding 100μg (20μl) of MTT to each well, incubating for three hours, decanting supernatant and finally adding 150 μl of dimethylsulphoxide to each well to dissolve the formazon. The plates were incubated for 15 minutes at 37°C and read spectrophotometrically at 540 nm; and cytotoxicity percentage was calculated by following formula:

Cytotoxicity Percentage = 100x [1-X/R1],

where X= (absorbance of the treated sample at 540 nm-absorbance of a blank at 540 nn) and

R1 = (absorbance of the untreated control at 540nm) - (absorbance of the blank at 540nm).

Thus in each of the MTT cytotoxicity assay the percentage was calculated according to the above formula and was based on the proliferation of the untreated controls, the value of which was considered as 100%.

EXAMPLE 12

The biological activity of synthesized peptide SEQ ID NO:3 was tested on different human tumor cell lines such as HT-29 & PTC (colon), A549 (non small lung cell), KB (oral squamous cell), MCF7 & MDA.MB.453 (Breast), HuTu80 (duodenum), PA-1 (ovary), MOLT-4 (leukemia) and MIAPaCa2 (Pancreas) at various molar concentrations. The percentage cytotoxicity induced by different

concentrations of the peptide SEQ ID NO: 3 is summarized in the following table.

	Percentage cytotoxicity at different concentrations					
Cell Line	1μM	100n M	10 nM	1M	100pM	10pM
MCF 7	Nil	Nil	24.35 ± 5	30.68 ± 6	38.95± 4.5	39.33 ± 2.6
MIAPaCa2	33.3 ± 4.5	30.3 ± 4.2	33.2 ± 6.7	36.4 ± 0.5	28.2 ± 4.5	27.4 ± 4.5
HuTu80	12.2 ± 4.0	15.5 ±4.7	14.3±3.5	13.3 ± 4.0	14.7 ± 4.2	10.3 ±3.5
KB	32.1 ± 5.0	31.6 ± 6.5	30.9±5.5	30.4 ± 6.5	26.4 ± 4.5	40.9 ± 5.5
A549	30.7 ± 6.5	23.6 ± 4.5	32.2±5.5	32.4 ± 4.5	25.2 ± 3.5	30.5 ± 3.5
HT29	25.4 ± 5.5	17.8 ± 4.5	11.8±5.0	20.3 ± 4.5	19.9 ± 5.5	18.7 ± 4.5
PTC	17.9 ± 2.5	27.7 ± 2.8	27.7±3.6	23.8 ± 2.8	26.5 ± 3.8	80.0 ± 7.1
MDA.MB.453	5.6 ± 3.5	11.2 ± 3.1	Nil	9.6 ± 1.9	25.5 ± 2.9	49.5 ± 4.2
PA-1	31.2 ± 5.1	34.2 ± 5.8	25.4±4.2	36.1 ± 6.1	40.1 ± 6.2	37.7 ± 3.9
MOLT-4	9.0 ± 1.2	1.4 ± 1.0	Nil	1.0 ± 0.4	15.9 ± 3.0	49.9 ± 4.1

EXAMPLE: 13

The cytotoxic activity of other synthesized bombesin analogs was tested on eight human tumor cell lines namely HT-29, SW620, PTC (all colon), PA-1 (ovary), A549 (lung), HBL100 (breast), MOLT-4 (leukemia) and DU145 (prostate). The tumor cells were collected at exponential growth phase and resuspended in medium (1.5×10^6) cells/ml in RPMI 1640 containing 10% FBS). 150μl of medium was added to the wells of a 96-well tissue culture plate (Nunc, Denmark) followed by 30μl of cell suspension. The plate was left in incubator (37°C, 5% CO_2 overnight. 20μl of the peptide ($10^{-7} \times 10^{-10}$ M concentration) was added to marked wells of the 96-well plate. Each concentration was plated in triplicates. 20μl of medium alone was added to control wells while wells without cells served as blanks. A total volume of 200μl was ensured in each well and plate was left in incubator (37°C, 5% CO_2). After 72 hours of incubation an MTT assay was performed and percentage cytotoxicity was calculated with respect to control cells. Following tables show the cytotoxicity achieved on various cell lines at different concentrations.

PA-1

S.No	Percent Cytotoxicity			
	100 nM	10nM	1 nM	100 PM
SEQ ID:4	2.3 ± 2.9	4.3 ± 0.2	16.2 ± 2.9	12.6 ± 2.9
SEQ ID:5	8.8 ± 1.9	20.9 ± 5.3	16.0 ± 3.9	25.6 ± 6.3
SEQ ID:6	9.2 ± 1.0	8.7 ± 1.9	7.4 ± 1.0	11.1 ± 2.9
SEQ ID:7	9.6 ± 4.1	22.7 ± 3.4	25.6 ± 2.9	24.5 ± 4.2
SEQ ID:8	10.4 ± 3.7	20.4 ± 3.0	23.8 ± 4.2	23.3 ± 5.5

PTC

S.No	Percent Cytotoxicity			
	100 nM	10nM	1 nM	100 pM
SEQ ID:4	9.8 ± 1.7	2.1 ± 0.2	8.7 ± 1.5	14.9 ± 1.1
SEQ ID:5	20.4 ± 4.2	15.9 ± 2.4	23.0 ± 4.2	13.9 ± 2.2
SEQ ID:6	24.7 ± 5.2	10.4 ± 0.8	9.1 ± 0.7	10.1 ± 0.6
SEQ ID:7	9.3 ± 1.8	7.6 ± 0.7	12.4 ± 2.1	8.2 ± 0.9
SEQ ID:8	8.7 ± 2.1	5.4 ± 1.7	12.5 ± 1.7	12.3 ± 1.9

DU145

S.No	Percent Cytotoxicity			
	100 nM	10nM	1 nM	100 pM
SEQ ID:4	24.9 ± 3.2	23.4 ± 3.3	22.8 ± 4.1	23.2 ± 3.7
SEQ ID:5	32.3 ± 3.8	22.0 ± 3.4	10.6 ± 0.9	29.3 ± 2.9
SEQ ID:6	13.7 ± 0.9	16.6±	3.9 ± 5.2	12.1 ± 0.8
SEQ ID:7	NIL	NIL	ND	ND
SEQ ID:8	19.1 ± 2.1	22.5 ± 2-2	21.4 ± 6.2	28.1 ± 3.5

SW620

S.No.	Percent Cytotoxity			
	100 nM	10 nM	1 nM	100 PM
SEQ ID: 4	34.3 ± 4.2	23.2 ± 2.0	27.8 ± 2.8	30.4 ± 3.2
SEQ ID: 5	25.6 ± 4.2	30.1 ± 4.0	29.7 ± 4.2	38.0 ± 3.8
SEQ ID: 6	23.5 ± 5.1	38.1 ± 7.3	33.5±5.2	24.8±4.2
SEQ ID: 7	25.4 ± 2.9	20.8 ± 1.9	32.0 ± 5.8	33.6 ± 5.8
SEQ ID: 8	29.4 ± 2.9	33.0 ± 3.8	20.6±3.9	20.6±3.9

HT29

S.No	Percent Cytotoxicity			
	100 nM	**10nM**	**1 nM**	**100 PM**
SEQ ID: 4	38.6 ± 5.3	38.9 ± 7.3	39.6 ± 4.3	43.3 ± 4.4
SEQ ID: 5	35.7 ± 2.8	44.4 ± 4.0	27.9 ± 2.9	42.0 ± 2.0
SEQ ID: 6	NIL	6.8 ± 0.7	26.7 ± 4.2	16.8 ± 0.5
SEQ ID: 7	15.5 ± 1.9	28.2 ± 2.8	ND	ND
SEQ ID: 8	34.8 ± 4.2	18.9 ± 4.2	34.7 ± 3.3	21.4 ± 3.1

MOLT4

S.No	Percent Cytotoxicity			
	100 nM	**10nM**	**1 nM**	**100 PM**
SEQ ID: 4	16.2 ± 0.6	28.7 ± 4.2	19.3 ± 1.8	28.5 ± 4.8
SEQ ID: 5	NIL	4.3 ± 0.6	6.4 ± 0.2	8.7 ± 0.6
SEQ ID: 6	NIL	20.4 ± 4.3	0.8 ± 0.1	11.0 ± 0.6
SEQ ID: 7	13.1 ± 0.3	NIL	NIL	ND
SEQ ID: 8	2.6 ± 0.1	12.8 ± 3.3	9.3 ± 0.2	16.6 ± 3.1

HBL

S.No	Percent Cytotoxicity			
	100 nM	10nM	1 nM	100 PM
SEQ ID: 4	25.0 ± 3.1	33.2 ± 5.2	30.6 ± 4.2	33.0 ± 3.6
SEQ ID: 5	19.4 ± 4.5	16.7 ± 3.6	31.6 ± 5.3	19.3 ± 2.7
SEQ ID: 6	17.0 ± 0.5	6.0 ± 0.4	1.2 ± 0.3	NIL
SEQ ID: 7	16.1 ± 3.9	7.0 ± 0.7	12.0 ± 0.7	4.0 ± 0.6
SEQ ID: 8	11.9 ± 2.1	14.4 ± 2.1	12.2 ± 1.9	12.1 ± 1.9

A549

S.No	Percent Cytotoxicity			
	100 nM	10nM	1 nM	100 PM
SEQ ID: 4	20.0 ± 2.2	20.6 ± 1.9	22.7 ± 2.9	20.7 ± 4.2
SEQ ID: 5	30.3 ± 4.3	22.2 ± 3.1	20.2 ± 4.2	25.2 ± 5.6
SEQ ID:6	1.9 ± 0.6	3.2 ± 0.1	13.0 ± 0.8	12.4 ± 0.7
SEQ ID:7	6.7 ± 2.0	17.9 ± 0.9	ND	ND
SEQ ID:8	21.7 ± 3.3	20.7 ± 2.2	19.7 ± 3.1	17.0 ± 2.7

EXAMPLE 14

The cytotoxic effect of peptide sequences SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, were studied by MTT assay which is based on the principle of uptake of MTT[3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide], a tetrazolium salt by the metabolically active cells where it is metabolized by active mitochondria into a blue colored formazan product which can be read spectrophotometrically. Tumor cells KB (oral squamous), HuTu80 (Stomach), PTC and SW620 (colon), U87MG (Glioblastoma), HBL 100 (Breast), HeP2 (laryngeal) and L132 (Lung) were incubated with the peptide analogs for 48 hours at 37°C in a 96-well culture plate, followed by the addition of 100 µg MTT and further incubation of 1 hour. The formazan crystals formed inside the cells were dissolved with a detergent comprising 10% Sodium dodecyl sulfate and 0.01 N HCl and optical density read on a multiscan ELISA reader. The optical

density was directly proportional to the number of proliferating and metabolically active cells. Percent cytotoxicity of peptide analogs is shown in the following Table.

SEQ ID: 9

Cell	Percentage cytotoxicity at different concentrations					
	1 μM	100n M	10 nM	1nM	100pM	10p M
KB	10.4 ± 1.6	20.8 ± 1.7	23.0 ± 2.1	32.6 ± 3.7	26.9 ± 2.9	20.6 ± 4.1
HuTu80	14.2 ± 0.6	13.5 ± 2.1	23.5 ± 2.9	28.0 ± 1.8	23.8 ± 2.8	19.5 ± 0.4
PTC	10.3 ± 0.9	19.5 ± 4.1	26.8 ± 3.8	25.6 ± 5.1	24.5 ± 3.9	22.4 ± 2.2
U87MG	10.0 ± 0.0	21.4 ± 0.1	20.0 ± 0.0	21.8 ± 0.1	11.9 ± 4.1	0.0 ± 0.0
SW620	21.6 ± 2.1	25.8 ± 2.8	33.2 ± 2.9	30.8 ± 0.6	28.9 ± 0.2	15.1 ± 0.3
HBL100	17.2 ± 0.4	22.4 ± 1.7	28.1 ± 0.6	34.1 ± 1.8	28.6 ± 2.2	17.2 ± 0.1
HeP2	21.6 ± 1.8	17.8 ± 0.3	28.5 ± 3.1	21.3 ± 2.2	14.6 ± 0.6	0.0 ± 0.0
L132	18.3 ± 2.9	25.9 ± 2.6	27.2 ± 3.1	30.5 ± 4.1	22.4 ± 0.8	0.0 ± 0.0

SEQ ID: 10

Cell	Percentage cytotoxicity at different concentrations					
	1 μM	100n M	10 nM	1nM	100pM	10p M
KB	16.5 ± 0.2	22.0 ± 1.1	27.3 ± 2.7	31.1 ± 4.1	25.0 ± 6.3	19.2 ± 2.9
HuTu80	17.2 ± 1.1	21.0 ± 2.0	20.6 ± 1.7	23.3 ± 2.8	22.9 ± 0.2	13.5 ± 0.8
PTC	28.4 ± 3.6	29.3 ± 3.2	32.5 ± 5.1	29.4 ± 2.9	21.6 ± 3.1	22.2 ± 4.9
U87MG	10.0 ± 0.0	15.0 ± 0.5	20.0 ± 0.0	25.6 ± 2.1	16.5 ± 0.5	11.6 ± 1.7
SW620	22.2 ± 2.1	19.4 ± 1.8	25.5 ± 2.8	22.4 ± 1.7	20.9 ± 0.6	16.7 ± 0.2
HBL100	18.5 ± 1.7	21.2 ± 1.7	32.9 ± 0.7	23.3 ± 1.6	16.6 ± 0.1	21.1 ± 0.7
HeP2	19.9 ± 1.5	26.3 ± 1.7	27.5 ± 2.8	27.2 ± 2.6	19.1 ± 0.6	1.7 ± 0.1
L132	22.4 ± 1.8	27.8 ± 2.1	27.5 ± 2.8	29.5 ± 2.8	29.4 ± 1.9	1.9 ± 0.2

SEQ ID: 11

Cell	Percentage cytotoxicity at different concentrations					
	1 μM	100n M	10 nM	1nM	100pM	10p M
KB	24.2 ± 1.2	31.9 ± 2.1	31.9 ± 3.1	33.1 ± 2.1	26.7 ± 5.1	21.6 ± 3.7
HuTu80	14.2 ± 0.1	20.0 ± 3.1	27.3 ± 2.7	30.5 ± 4.1	22.6± 3.9	17.6 ± 1.6
PTC	18.6 ± 1.5	25.8 ± 2.5	25.7 ± 4.1	28.5 ± 2.8	28.3 ± 0.8	19.7 ± 0.6
U87MG	1.0 ± 0.1	15.5 ± 0.6	20.0 ± 0.0	24.2 ± 1.7	26.5 ± 2.6	21.9 ± 2.1
SW620	23.7 ± 1.4	21.0 ± 1.5	31.5 ± 2.6	35.1 ± 2.2	25.9 ± 3.8	20.4 ± 0.3
HBL100	24.5 ± 0.8	22.7 ± 0.5	29.9 ± 0.3	24.3 ± 1.6	15.4 ± 4.1	18.2 ± 1.1
HeP2	21.9 ± 2.1	23.9 ± 1.1	34.6 ± 2.2	37.1 ± 3.3	20.1 ± 0.0	15.1 ± 0.3
L132	1.4 ± 1.1	20.4 ± 1.5	30.4 ± 0.4	29.4 ± 0.4	18.3 ± 0.9	0.5 ± 0.0

SEQ ID: 12

Cell	Percentage cytotoxicity at different concentrations					
	1 μM	100n M	10 nM	1nM	100pM	10p M
KB	12.4 ± 1.2	11.1 ± 3.1	18.6 ± 2.1	26.6 ± 4.9	19.4 ± 2.9	19.3 ± 2.9
HuTu80	20.0 ± 3.9	21.8 ± 2.1	23.4 ± 0.5	33.1 ± 4.8	13.0 ± 0.7	8.3 ± 1.1
PTC	14.4 ± 2.7	16.1 ± 2.5	20.7 ± 3.8	30.1 ± 4.1	18.6 ± 2.4	19.5 ± 0.8
U87MG	15.4 ± 3.1	13.1 ± 2.3	27.5 ± 2.9	28.3 ± 1.9	22.1 ± 3.8	13.1 ± 2.2
SW620	22.6 ± 1.1	25.3 ± 0.6	36.1 ± 1.9	32.2 ± 2.6	38.4 ± 2.8	34.8 ± 0.4
HBL100	11.8 ± 1.1	23.6 ± 2.7	27.7 ± 1.5	29.6 ± 0.4	34.7 ± 2.8	29.0 ± 3.8
HeP2	28.7 ± 0.8	25.6 ± 0.4	29.2 ± 1.1	28.9 ± 0.5	24.4 ± 0.1	10.0 ± 0.0
L132	22.2 ± 0.2	22.0 ± 0.1	26.4 ± 0.3	26.7 ± 0.4	23.1 ± 0.7	0.0 ± 0.0

All publications referenced are incorporated by reference herein, including the nucleic acid sequences acid sequences and amino acid sequences listed in each publication. All the compounds and methods disclosed and referred to in the publications mentioned above are incorporated by reference herein, including those compounds disclosed and referred to in articles cited by the publications mentioned above.

CLAIMS

1. A peptide of the formula

X-D-Phe-Gln-R1-R2-Val-R3-His-R4-NH_2

wherein X is acetyl or straight, branched or cyclic alkanoyl group from 3-16 carbon atoms, or X is deleted

R1 is Trp or D-Trp,

R2 is Ala, Aib or Deg,

R3 is Gly, Aib, Deg, Dpg or Ac5c,

R4 is Leu or Ile

or a hydrolyzable carboxy protecting group; wherein at least one of R2 or R3 is an α,α- dialkylated amino acid; or a pharmaceutically acceptable salt of the peptide.

2. The peptide of claim 1, wherein X is deleted, R1 is Trp, R2 is Ala, R3 is Aib and R4 is Leu, and said peptide has the formula:

D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO: 3)

or a pharmaceutically acceptable salt thereof.

3. The peptide of claim 1, wherein X is deleted, R1 is Trp, R2 is Aib, R3 is Gly and R4 is Leu, and said peptide has the formula:

D-Phe-Gln-Trp-Aib-Val-Gly-His-Leu-NH_2 (SEQ ID NO: 4)

or a pharmaceutically acceptable salt thereof.

4. The peptide of claim 1, wherein X is deleted, R1 is D-Trp, R2 is Ala, R3 is Aib and R4 is Leu, and said peptide has the formula:

D-Phe-Gln-D-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO:5)

or a pharmaceutically acceptable salt thereof.

5. The peptide of claim 1, wherein X is deleted, R1 is Trp, R2 is Aib, R3 is Gly and R4 is Ile, and said peptide has the formula:

D-Phe-Gln-Trp-Aib-Val-Gly-His-Ile-NH_2 (SEQ ID NO: 6)

or a pharmaceutically acceptable salt thereof.

6. The peptide of claim 1, wherein X is deleted, R1 is Trp, R2 is Ala, R3 is Aib and R4 is Ile, and said peptide has the formula:

D-Phe-Gln-Trp-Ala-Val-Aib-His-Ile-NH_2 (SEQ ID NO:7)

or a pharmaceutically acceptable salt thereof.

7. The peptide of claim 1, wherein X is deleted, R1 is D-Trp, R2 is Ala,

R3 is Dpg and R4 is Leu, and said peptide has the formula:

D-Phe-Gln-D-Trp-Ala-Val-Dpg-His-Leu-NH_2 (SEQ ID NO:8)

or a pharmaceutically acceptable salt thereof.

8. The peptide of claim 1, wherein X is deleted, R1 is Trp, R2 is Deg, R3 is Gly and R4 is Leu, and said peptide has the formula:

D-Phe-Gln-Trp-Deg-Val-Gly-His-Leu-NH_2 (SEQ ID NO:9)

or a pharmaceutically acceptable salt thereof.

9. The peptide of claim 1, wherein X deleted, R1 is Trp, R2 is Ala, R3 is Ac5c and R4 is Leu, and said peptide has the formula:

D-Phe-Gln-Trp-Ala-Val-Ac5c-His-Leu-NH_2 (SEQ ID NO: 10)

or a pharmaceutically acceptable salt thereof.

10. The peptide of claim 1, wherein X is butanoyl, R1 is Trp, R2 is Ala, R3 is Alb and R4 is Leu, and said peptide has the formula:

Butanoyl-D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO: 11)

or a pharmaceutically acceptable salt thereof.

11. The peptide of claim 1, wherein X is octanoyl, R1 is Trp, R2 is Ala, R3 is Alb and R4 is Leu and said peptide has the formula:

Octanoyl-D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO: 12)

or a pharmaceutically acceptable salt thereof.

12. A composition comprising an effective amount of a polypeptide according to claim 1, and a pharmaceutically acceptable carrier.

13. A method of treatment of cancer in mammals which comprises the administration of an effective amount of a peptide according to claim 1.

14. A method according to claim 11, further comprising administering a chemotherapeutic compound.

15. A solid phase synthesis process for the preparation of a peptide analog of formula (I):

X-D-Phe-Gln-R1-R2-Val-R3-His-R4-NH_2

wherein X is acetyl or straight, branched or cyclic alkanoyl group from 3-16 carbon atoms, or X is deleted,

R1 is Trp or D-Trp,

R2 is Ala, Aib or Deg,

R3 is Gly, Aib, Deg, Dpg or Ac5c,

R4 is Leu or Ile

which comprises sequentially loading protected α,α-dialkylated amino acids in sequential cycles to the amino terminus of a solid phase resin, coupling the amino acids to assemble a peptide-resin assembly, removing the protecting groups and cleaving the peptide from the resin to obtain a peptide.

16. The process as claimed in claim 13, wherein said α-, α-dialkylated amino acids are protected at their α-amino groups by a 9-fluorenyl methoxy carbonyl (Fmoc) group.

17. The process as claimed in claim 15, wherein the coupling is carried out in the presence of activated agents selected from the group consisting of DCC, DIPCDI, DIEA, BOP, PyBOP, HBTU, TBTU, and HOBt.

18. The process as claimed in claim 15, wherein the coupling is carried out in the presence of a solvent selected from the group consisting of DMF, DCM, and NMP or a mixture thereof.

19. The process as claimed in claim 15, wherein said peptide is cleaved from said peptide-resin assembly by treatment with trifluoroacetic acid, crystalline phenol, ethanedithiol, thioanisole and water for 1.5 to 5 hours at room temperature.

20. The process as claimed in claim 15, wherein the α, α-dialkylated amino acid is prepared by conversion of a ketone to a hydantoin and hydrolysis of said hydantoin.

ABSTRACT

The present invention encompasses novel peptides that are antagonists to bombesin and bombesin like peptides and are useful in the treatment of cancer. The invention particularly relates to the design and synthesis of the novel peptides incorporating α,α-amino acids in a site specific manner. The invention encompasses methods for the generation of these peptides, compositions containing the peptides and the pharmacological applications of these peptides especially in the treatment and prevention of cancer.

SEQUENCE LISTING

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PRASAD, SUDHANHAND
MUKHERJEE, RAMA
JAGGI, MANU
SINGH T, ANU
MATHUR, ARCHNA

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